gesting that cells present in this compartment post-BMT may be composed of both migrants and a resident population. To mediate resistance to PC engraftment, effector CD8+ cells must first survive conditioning and the BMT milieu. B6BALB.B mice irradiated at 3, 6, and 9 Gy were analyzed 24 hours later for CD8+ H60-specific T cells in the spleen and BM. Tertramer+ cells were clearly identified in both compartments showing dose-dependent increases. Importantly, 5 days after HCT with 1×10^7 H60 congenic (or BALB.B) TCD BM, PC rejection was evident, as indicated by nondetectable splenic CFU activity in recipients of these allogeneic, but not syngeneic TCD-BM. Notably, tetramer+ cells were again detected in BM and spleen. Interestingly, their frequency was 3fold higher in the marrow (mean, 6%) than in the splenic compartment (mean, 1.7%) in sensitized recipients of either syngeneic or MiHA allogeneic BM. These increases are consistent with the predicted survival advantage of CD8+ memory T cells due to elevated bcl-2 levels. However, resistance did not occur in syngeneic recipients due to lack of activation of H60-specific T cells. These studies conclusively demonstrate that an antigen-specific CD8 T-memory population responsible for resistance survives allogeneic BMT and is present in bone marrow and spleen where resistance is presumed to occur. Studies are underway to functionally and molecularly characterize this MiHA antigen-specific population during ongoing resistance post-HCT.

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IMMUNOLOGICAL AUTOLOGOUS STEM CELL GRAFT ENGINEERING USING COMBINATION G-CSF AND ALDESLEUKIN (IL-2) IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Absolute lymphocyte count (ALC) recovery to ≥ 500/μl by day 15 post-autologous peripheral blood stem cell transplant (APBSCT) is associated with an improved overall and event-free survival (EFS) for patients with non-Hodgkin's lymphoma (NHL). The benefit of the ALC post-APBSCT is likely due to natural killer (NK) cells, because patients who achieved a normal NK cell count (≥ 80/μl) on day 15 post-APBSCT (day 15 NK) had an improved EFS compared with those whose day 15 NK was low. Finally, the day 15 ALC correlates to the infused autograft absolute lymphocyte count (A-ALC) and not with total CD34 dose. Thus we hypothesized that a peripheral blood stem cell mobilization regimen would be ideal if it could mobilize CD34+ cells, lymphocytes, and NK cells. A pilot study was undertaken using a sequential mobilization regimen of granulocyte colony-stimulating factor (G-CSF) followed by aldesleukin. Patients received G-CSF at 10 μg/kg/day, and stem cell collections began on day 5 or later, when the peripheral blood (PB) CD34 cell count was ≥ 10/μl. Once stem cell collection was complete and a minimum of 2×10^6 CD34 cells/kg were collected, patients received 1 subcutaneous injection of aldesleukin and underwent 1 further apheresis the next day collect lymphocytes. The dose levels of aldesleukin were 0, 0.5, 1, 1.5, and 2×10^6 U/m^2. Patients then underwent conditioning for APBSCT and on day 0 received all of the apheresis product, including the extra collection of lymphocytes. PB lymphocyte counts and subset analysis were measured at baseline (before G-CSF), pre-aldesleukin, 1 day post-aldesleukin, and day 15 post APBSCT. A total of 43 patients with NHL were entered in the study. Of these, 26 completed an adequate CD34 collection, received aldesleukin, and underwent an extra apheresis. The results of these 26 patients are presented. The median CD34 collected, A-ALC, autograft NK collected (A-NK), day 15 ALC, and day 15 NK for each dose level are given. The median times to neutrophil and platelet engraftment were 13 and 12 days, respectively. We conclude that aldesleukin up-regulates NK cell numbers. Dose level 3 resulted in the best NK cell apheresis collection, the highest PB NK cell count post-aldesleukin, on day 15 NK, and on day 15 ALC. Only at this dose did all patients achieve a normal day 15 NK cell count. A phase II study is proposed using a sequential regimen of G-CSF followed by aldesleukin at a dose of 1.5×10^6/m^2 to confirm these results. Successful lymphocyte and NK cell engraftment post-APBSCT may become as important as neutrophil and platelet engraftment post-APBSCT.

Dose Level	IL-2 Dose	# Patients	CD34	Total A-ALC	Total A-NK	Day 15 ALC	Day 15 NK
0	0	5	7.36×10^6/kg	0.57×10^9/kg	0.09×10^9/kg	744/μl	194/μl
1	0.5×10^6 IU/m^2	5	4.37×10^6/kg	0.47×10^9/kg	0.09×10^9/kg	310/μl	92/μl
2	1×10^6 IU/m^2	5	4.87×10^6/kg	0.67×10^9/kg	0.10×10^9/kg	700/μl	120/μl
3	1.5×10^6 IU/m^2	5	6.96×10^6/kg	0.38×10^9/kg	0.11×10^9/kg	1380/μl	472/μl
4	2×10^6 IU/m^2	6	3.73×10^6/kg	0.43×10^9/kg	0.10×10^9/kg	787/μl	192/μl

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INHIBITORY EFFECTS OF CYTOTOXICITY OF CYTOKINE ACTIVATED AND EXPANDED HUMAN CD8+ T CELLS BY A HUMAN NKG2D ALTERNATIVE SPLICE VARIANT

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NKG2D is an activating receptor that is expressed on CD8+ T cells and γ&δ T cells where it up-regulates activation through T-cell receptor (TCR) costimulation. NKG2D is triggered by ligands that are structurally related to major histocompatibility complex (MHC)-1 and expressed by tissues early in ontogeny, stress, and transformation. The function of NKG2D on natural killer (NK) cells and CD8+ T cells is mediated by 2 distinct pathways of signaling through its association with DAP10 and DAP12. One functionally distinct human NKG2D isoform has been identified. In evaluating human NKG2D gene expression by reverse-transcription polymerase chain reaction (RT-PCR) in CD8+T cells derived from 7 different donors, we consistently observed 2 distinct bands differing by 218bp. Cloning and sequencing of both PCR amplicons revealed a previously described alternatively spliced NKG2D variant from inclusion of intron 6. Intron 6 inclusion introduces a stop codon, resulting in a truncated protein product lacking the entire extracellular domain. To determine the functional consequences of truncated NKG2D expression, we isolated and cloned both full-length and truncated NKG2D into pRJ3 vectors. Human CD8+ T cells were tranduced and directed against human cell line targets in Cr^{51} cytotoxicity assays. We found that expression of the full-length NKG2D resulted in a ~15% increase in cytotoxicity, whereas expression of the truncated NKG2D resulted in ~70% reduction in cytotoxicity. Results were compared to CD8+ T cells transduced with an empty vector. To further elucidate the role of each NKG2D isoform, we coexpressed full-length and truncated NKG2D with DAP-10 or DAP-12. Coexpression of full-length NKG2D with either DAP10 or DAP12 resulted in a comparable 25% cytotoxicity versus NKG2D alone. Coexpression of full-length and truncated NKG2D also resulted in reduction of up to 65% coexpression of the truncated NKG2D isoform with DAP10 or DAP12 did not alter the reduction in cytotoxicity observed with truncated NKG2D alone. We also performed experiments using murine CD8+ T cells with human cell line targets. Overexpression of full-length NKG2D resulted in a > 32% increase in cytotoxicity, whereas coexpression of full-length NKG2D and DAP10 resulted in a 42% increase in cytotoxicity. Collectively, these results demonstrate that the NKG2D isform lacking the extracellular domain unexpectedly inhibits cytotoxicity in human CD8+ T cells. Also of interest is whether variability in expression of the truncated NKG2D isoform influences the cytotoxic potency of activated CD8+ T cells expanded from different donors.

GVH/GVL

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RABBIT IGG LEVELS IN PATIENTS RECEIVING THYMOGLOBULIN AS PART OF CONDITIONING BEFORE UNRELATED-DONOR ALLOGENEIC STEM CELL TRANSPLANTATION

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Purpose: Thymoglobulin (ATG) given before allogeneic hematopoietic stem cell transplantation (HSCT) with unrelated donors reduces acute graft-versus-host disease (GVHD). However, the possible role of serum concentration of rabbit ATG for the subsequent development of acute GVHD after HSCT is unknown. **Methods:** The serum concentration of rabbit IgG was analyzed by enzyme-linked immunosorbent assay in 61 patients after unrelated donor HSCT. Doses of ATG as part of the conditioning ranged between 4 and 10 mg/kg. Stem cell source was bone marrow (BM) in 28 cases and peripheral blood (PBSC) in 33. Conditioning was mainly cyclophosphamide combined with total body irradiation (TBI) or busulfan. Most patients received GVHD prophylaxis with cyclosporine and methotrexate. **Results:** Even though we found a good correlation between given ATG dose and serum concentration of rabbit IgG after transplant ($r = 0.67$), there was a wide variation of rabbit IgG levels within each dose group. After administration, levels of rabbit IgG decreased slowly and could still be detected up to 5 weeks after HSCT. We found a correlation between grade of acute GVHD and concentration of rabbit IgG in serum obtained before transplantation ($P = .017$). Patients with serum levels of rabbit IgG > 70 mg/ml before HSCT had very low risk for developing acute GVHD grades II-IV compared with those with < 70 mg/ml (11% vs 53%; $P < .001$). **Conclusion:** Measuring rabbit IgG levels in patients receiving ATG as prophylaxis against GVHD after HSCT may be a helpful tool in decreasing the risk of severe GVHD.

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GENERATION OF TOLEROGENIC DENDRITIC CELLS AND REGULATORY T CELLS THROUGH INTRAVENOUS DELIVERY OF AUTOLOGOUS PHOTOPHERESIS-INDUCED APOPTOTIC CELLS

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Phagocytosis of apoptotic cells by macrophages or dendritic cells has been shown to regulate immune responses both in vivo and in vitro. Extracorporeal photopheresis (ECP) involves the clinical reinfusion of peripheral blood leukocytes that are undergoing apoptosis following exposure ex vivo to 8-methoxypsoralen (8-MOP) and UVA light. ECP is approved for the palliative treatment of cutaneous T-cell lymphoma and has been reported to have utility in immune-mediated inflammatory diseases, such as graft-versus-host disease (GvHD) and solid-organ transplant rejection, and autoimmune diseases, such as rheumatoid arthritis and Crohn's disease. We have evidence to suggest that ECP therapy may modulate host dendritic cell function and induce regulatory T-cell generation. When coincubated with ECP-treated cells, activated dendritic cells produce reduced levels of proinflammatory cytokines, such as interleukin-12, whereas transforming growth factor-β levels are modestly increased. Activation of CD4+ T cells in the presence of allogeneic dendritic cells and ECP-treated cells promotes generation of a population of T cells that can suppress proliferation of naive syngeneic T cells, as well as suppress interferon-γ production. To confirm these findings in vivo, we used a murine contact hypersensitivity model. ECP-treated or control leukocytes from mice sensitized with the hapten dinitrofluorobenzene (DNFB) were injected intravenously into naive recipients. Compared with controls, mice that received ECP-treated cells demonstrated significantly less ear swelling after sensitization and challenge with DNFB. Suppression of ear swelling was specific for DNFB and was cell-mediated, as demonstrated by the ability to transfer DNFB tolerance to naive mice, which could appropriately respond to the unrelated hapten oxazalone. Transfer of this tolerance was abrogated by depletion of either CD4+ or CD25+ T-cell populations. Collectively, these results suggest that the delivery of ECP-treated cells promotes generation of regulatory T cells that are capable of modulating immune responses. Regulatory T cells have been implicated in the control of GvHD, and as previously reported, ECP has demonstrated beneficial activity in GvHD. As a result, international phase II clinical trials are currently underway to assess the efficacy of photopheresis in GvHD patients.

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PRETRANSPLANT RECIPIENT BLOOD CD14+ PREDC LEVELS CORRELATE WITH INCREASED ACUTE GVHD AFTER ALLOGENEIC PBSC TRANSPLANTATION

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Host dendritic cells (DCs) present host alloantigens to donor T lymphocytes. Human peripheral blood contains various circulating DC precursors including CD11c+ myeloid preDC (mDC), CD14+ monocytic DC precursors (CD14+ preDC) and plasmacytoid preDC (pDC). We used flow cytometry to enumerate both mDC (lin-, HLA-DR+, and CD11c+), mono-DC (CD14+), and pDC (lin-, HLA-DR+, and CD123+) numbers in the blood of patients receiving an allogeneic HSCT. Fifty consecutive patients undergoing HSCT from HLA-matched related (n = 28) or unrelated (n = 22) donors were enrolled in the study. The stem cell source was bone marrow in all unrelated donors, and branulocyte colony-stimulating factor (G-CSF) mobilized PBSC in related donors. All patients received CsA and MTX as GVHD prophylaxis. Moreover, 26 patients (52%) received ATG before transplant. mDC and pDC PB counts were significantly lower in patients than in 28 age-matched healthy controls (8.8 cells/μl [25th-75th percentile, 3.5–14.5] mDC, and 2.8 [1.3–5.5] pDC, versus 15.5 [12.1–25.1] and 8.6 [5.6–13.1], respectively; $P < .001$). However the mDC/pDC ratio was significantly higher in the patient group (3.5 [1.6–6.2] vs 1.7 [1.3–2.6]; $P = .002$). CD14+ preDC counts were not significantly different. Among the 46 patients who were evaluable, 12 (26%) developed acute GVHD grade II-IV. Risk factors significantly associated with acute GVHD were older age ($P = .01$), PBSC transplantation ($P = .02$), and the absence of ATG in the conditioning regimen ($P = .01$). Patients with acute GVHD had significantly higher pretransplantation mDC:pDC ratio (5.7 [3.3–16.4] vs 3.1 [1.6–5.5]; $P = .03$) and CD14+ preDC counts (395 [326–625] vs 284 [187–395]; $P = .02$). A subset analysis was performed in PBSC patients, only 3 of whom had received ATG before transplant. Among 26 evaluable patients, 10 (38%) developed acute GVHD grade II-IV. Besides older age ($P = .02$), the only risk factors significantly associated with acute GVHD in PBSC patients were the pretransplantation mDC:pDC ratio (5.7 [4.1–13.1] vs 1.7 [1.2–2.9]; $P = .008$) and CD14+ preDC counts (395 [352–710] vs 259 [199–314]; $P = .004$). In multivariate analysis, only older age ($P = .04$) and pretransplantation circulating CD14+ preDC numbers ($P = 0.04$) were significantly associated with acute GVHD in PBSC transplants. These findings demonstrate that blood levels of DC precursors may correlate with a higher risk of developing aGVHD. Future studies will be aimed at depleting host preDC before allotransplant as a means of GVHD prophylaxis.

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ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD) IN PATIENTS UNDERGOING ALLOGENEIC BMT FOR THALASSAEMIA MAJOR

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Methods: Between October 1991 and June 2004, 152 thalassemic patients who underwent allogeneic BMT and survived more than 2 weeks were evaluated for graft-versus-host disease (GVHD). Conditioning regimens included cusulfan (Bu) 16 mg/kg, cyclophosphamide (Cy) 200 mg/kg, and antilymphocyte globulin (ALG) 120 mg/kg (in 91 patients); Bu 600 mg/m^2 and Cy 200 mg/kg (in 51 patients), Bu 14 mg/kg, Cy 10 mg/kg, and ALG 120 mg/kg (in 8 patients), and others (in 2 patients). GVHD prophylaxis consisted of cyclosporine (CSA) alone in 11 patients, CSA and methotrexate (MTx) (15 mg/m^2 on day 1, 10 mg/m^2 on days 3, 6, and 11) in 40 patients and CSA and MTx (10 mg/m^2 on day 1, 7 mg/m^2 on days 3, 6, and 11) in 99 patients. All donors were 6 antigen HLA-matched sibling (91.4%) or family (8.6%) donors. **Results:** There were 103 males and 49 females, with mean age of